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CHRONIC INFECTIONS IN A FAMILY WITH HEREDITARY DEFICIENCY OF IgG2 AND IgG4

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SUMMARY

Two siblings, a girl and a boy, and their mother were abnormally susceptible to infections with bronchopneumonias leading to chronic pulmonary atelectasis in the girl and otitis leading to necrotizing changes with deafness in the mother. The causative micro-organism most often found was *Haemophilus influenzae*. The serum IgG was within the normal concentration range, but showed restricted electrophoretic heterogeneity with absence of the anodal component. Serum IgG2 and IgG4 were absent. Absence of antibodies to teichoic acid and haemagglutinating antibodies to *Haemophilus influenzae* polysaccharide was noted as was the case with isohaemagglutinins and heteroagglutinins against sheep cells. Lymphopenia and poor [^{3}H]thymidine uptake by peripheral lymphocytes after PHA stimulation were demonstrable in the girl and the mother. The girl had developed an epithelial ovarian tumour. The disease was a non-X-linked recessive and expressed selective humoral and selective cellular immunodeficiency. An inherited structural or regulatory gene defect is suggested. Gamma-globulin therapy had a good clinical effect.

INTRODUCTION

Immunodeficiency syndromes are gradually being recognized in an increasing range of combinations of qualitative and quantitative defects of both humoral and cellular immunity. Several well-defined clinical entities have been described (*Bull. Wld Hlth Org.*, 1971). Since the description of the four IgG subclasses determined by structural differences located on the heavy chains (Grey & Kunkel, 1964; Terry & Fahey, 1964) a variation and imbalance of IgG subclasses have occasionally been found (Terry 1968; Rivat *et al.*, 1969; Schur *et al.*, 1970; Yount *et al.*, 1970). The occurrence of independent genetic mechanisms controlling the synthesis of the heavy chains of each IgG subclass has been demonstrated by Rivat and coworkers (1970).

A family in which members had recurrent *Haemophilus influenzae* infections with chronic pulmonary atelectasis and chronic otitis with deafness was studied and found to have a selective humoral and selective cellular immune defect.

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CASE REPORTS

Y.W.

A girl, born April 10, 1959, the first of three siblings, was admitted to hospital in April 1972. During her 1st year of life she gained weight very badly. At about 3 years of age she had pyelitis. From infancy onwards she had repeated otitis media. In the last few years she had also suffered from coughing, with yellow–green sputum, which did not respond to antibiotic therapy.

Physical examination: poor inspiration sound over the left lung. Lymph nodes sometimes enlarged. Tonsils small. May 5, 1972, the patient was operated upon because of an ovarian tumour. The tumour, an encapsulated granulosa cell tumour ($7{\cdot}5 \times 10 \times 13{\cdot}5$ cm), was radically removed. No signs of metastasis.

Laboratory findings: ESR 9 mm/hour, Hb 14·2 g/100 ml. Red blood cells 5·1 million/mm^3. Reticulocytes 9‰, platelets 130,000/mm^3. Leucocytes 4700/mm^3, with band-shaped neutrophils 9%, segmented neutrophils 39%, eosinophils 20%, basophils 1%, lymphocytes 19%, monocytes 12%. Culture of the sputum showed *Haemophilus influenzae* on most occasions.

Bone marrow specimen: few plasma cells. Normal lymphocytes. Chest X-ray: atelectasis of the lower and middle lobes of the left lung. Lymph node biopsy: microscopy showed a normal picture with several lymph follicles with germinal centres and numerous lymphoid cells in the cortex and paracortically. Rectal biopsy: microscopic examination revealed normal findings with several lymph follicles with germinal centres and normal plasma cells. Biopsy of ovarian tumour: the tumour with capsule was classified as a granulosa cell tumour with atypical cells and mitosis.

R.W.

A boy, born March 3, 1966, the third of three siblings, half brother to the described patient Y.W., with the same mother, was admitted to hospital in April 1972. During the first year of life he had had several attacks of otitis media and infections of the upper respiratory tract. He had mostly suffered from recurrent otitis media. Since 4 years of age recurrent bronchopneumonia had occurred and despite adequate antibiotic therapy chest X-ray showed irreversible changes. Physical examination: the patient is between the third and tenth percentile for height and weight. Lymph nodes often enlarged. Tonsils normal size.

Laboratory findings: ESR 4 mm/hour. Haemoglobin 14·5 g/100 ml. RBC 4·5 million/mm^3. Reticulocytes 7‰, platelets 220,000/mm^3. Leucocytes 4700/mm^3, with band-shaped neutrophils 3%, segmented neutrophils 36%, eosinophils 3%, lymphocytes 52%, monocytes 6%. Culture of nasal secretion almost regularly showed growth of *Haemophilus influenzae*. Bone marrow specimen: few plasma cells. The lymphocytes had small hyperchromatic nucleus without cytoplasm. Increased number of reticulum cells with signs of erythrocyte phagocytosis. Lymph node biopsy after regional stimulation: normal findings. Rectal biopsy: normal findings.

S.W.

The mother, born 1936, had for several years suffered from recurrent otitis media resulting in chronic necrotizing otitis and deafness of the right ear. She had had bronchopneu-

monia two to three times per year. She has chronic bronchitis. Culture of nasal secretions often gave growth of *Haemophilus influenzae*.

FAMILY STUDIES

The girl Y.W. is number one of three siblings. Her father, K.R.W., was investigated and is healthy. Number two of the siblings, L.W., is healthy. The boy R.W. is number three of the three siblings. L.W. and R.W. are said to have the same father, who was not available for the investigation. The mother S.W. has one half sister (the same mother), and she is well. The grandmother is well. The father of S.W. is unknown.

MATERIALS AND METHODS

Serum samples were obtained at intervals from the three patients and their relatives. The samples were kept at −20°C until analysed.

Anti-human IgG, IgA, IgM, IgD, kappa chains and lambda chains

These were used as previously described (Oxelius, 1972).

Anti-human IgG2 and IgG4

Rabbits were made tolerant with Gm(1) kappa and Gm(4) lambda myeloma proteins and afterwards immunized with IgG2 myeloma proteins and IgG4 myeloma proteins, respectively, according to the technique described by Spiegelberg & Weigle (1968). The resultant antisera were absorbed with appropriate myeloma proteins. On immunelectrophoresis and double diffusion they identified respectively with an anti-IgG2 antiserum (kindly placed at our disposal by Professor H. G. Kunkel, New York) and an anti-IgG4 antiserum (kindly supplied by Dr Skvaril, Berne, Switzerland).

Anti-human IgG3

This was kindly supplied by Dr Yount, New York.

Gm antigenic determinations

These were performed according to the standard method adapted in microtitre system (Grubb, 1956; Borel *et al.*, 1967). Reagents for Gm(1), Gm(4) and Gm(5) determinations were kindly supplied by Professor Grubb, Lund, Sweden.

Quantitation of IgG, IgA, IgM, IgD, kappa chains, lambda chains, IgG2 and IgG4

This was performed by the single diffusion in tube technique of Oudin (1952) as modified by Bachmann & Laurell (1965) and by the electroimmunoassay (Laurell, 1966; Laurell, 1972).

Crossed immunoelectrophoresis and Agarose electrophoresis

These were performed according to Laurell (1965).

Immunoelectrophoresis

This was performed according to Scheidegger (1955).

Double diffusion in gel

This was performed according to Ouchterlony (1958).

Indirect haemagglutination test

This was performed according to Boyden (1950) adapted to a microtitre system, using sheep red cells coated with a preparation *Haemophilus influenzae polysaccharide.*

Haemagglutination of sheep cells (Forssman, 1911)

This was modified for use in a microtitre system.

Anti-teichoic acid

This was assayed using pepsin-treated extracts of *Staphylococcus aureus* strain Wood 46 in precipitation techniques in gel, immunelectrophoresis and double diffusion.

The culture of peripheral lymphocytes

This was continued for 76 hr with increasing amounts of phytohaemagglutinin, and the uptake of titriated thymidine was measured over the last 4 hr, calculating the dose–response curve according to the technique of Fitzgerald (1971).

TABLE 1. Quantitative determinations of immunoglobulin classes and IgG subclasses IgG2 and IgG 4 of serum of the patients

		Percentage of a normal serum pool (400 adult blood donors)					
		Class of immunoglobulin				IgG subclasses	
Patient	age	IgG	IgA	IgM	IgD	IgG2	IgG4
Y.W.	13 years	70	32	123	<22	<1	<1
R.W.	6 years	76	26	45	<22	<1	<1
S.W.	36 years	88	77	96	140	<1	<1
Mean normal value ± 1 SD (or range for IgD and IgG4) (14–19 controls in each group)	7–8 years	75±27	54±23	85±36	49 (<22–125)	72±28	73 (21–160)
	13–14 years	66±17	54±30	80±22	98 (<22–250)	70±18	75±31

RESULTS

Humoral immunity

Quantitation of IgG, IgA, IgM, IgD and kappa and lambda chains. In both children Y.W. and R.W. the concentrations of serum IgG and IgA were normal. IgM was occasionally low in R.W. but normal in patient Y.W. IgD was below detectable amount in serum in both Y.W. and R.W. Serum IgG, IgA, IgM and IgD were normal in the mother S.W. (Table 1). In repeated serum samples of the two children the IgA and IgM levels showed the normal tendency of increasing values with increasing age. Serum kappa/lambda quotients of all

three patients were normal. All of the relatives investigated showed normal levels of IgG, IgA and IgM.

Electrophoretic mobility of immunoglobulins. A restricted electrophoretic heterogeneity of the serum IgG was confirmed by immunelectrophoresis and crossed immunelectrophoresis (Fig. 1), which showed the bulk of IgG on the cathodal side of the application hole in all three patients. Furthermore, the kappa and lambda chains migrated cathodally (Fig. 1). The immunelectrophoretic pattern of IgA and IgM were normal.

IgG subclasses and Gm genetic markers. The IgG2 band and IgG4 band are absent in sera of the patients Y.W. and R.W., and their mother S.W., when tested in immunelectrophoresis with an anti-IgG2 antiserum and an anti-IgG4 antiserum (Fig. 1). Quantitation by the

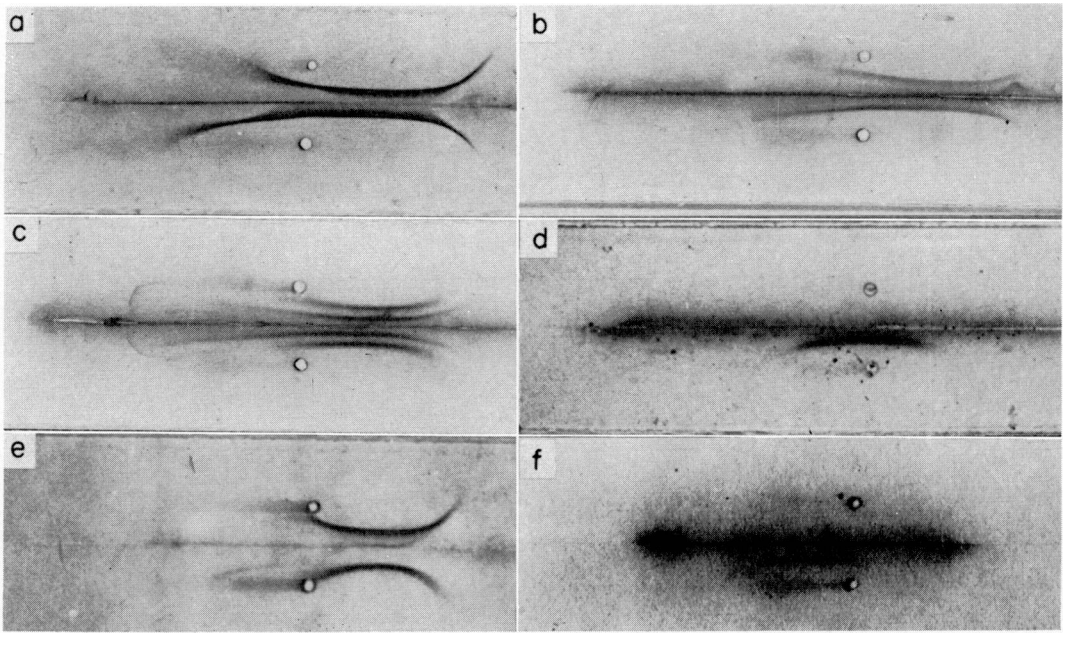

FIG. 1. Immunoelectrophoresis of serum of patient Y.W. (above) and normal serum as reference (below). The troughs contained (a) anti-IgG, (b) anti-kappa chains, (c) anti-lambda chains, (d) anti-IgG2, (e) anti-IgG3 and (f) anti-IgG4, respectively. A deficiency of anodal IgG is seen. The IgG2 band and the IgG4 band are missing in the serum of the patient. Patients R.W. and S.W. showed the same immunoelectrophoretic patterns.

Oudin technique of IgG2 and IgG4 showed levels below 1% of the amount in a normal serum pool of adults (Table 1). The relatives investigated showed normal levels of IgG2 ranging from 62% to 100% of a normal serum pool and of IgG4 from 75% to 100% of a normal serum pool. The IgG3 of the patients' sera appeared normal on immunelectrophoresis when compared with the normal serum pool or individual normal sera (Fig. 1).

No Gm(1) was detectable in the sera of Y.W., R.W. and S.W. A strong inhibitory effect was noted in the haemagglutination inhibition test for Gm(4) and Gm(5) indicating that Y.W. and R.W., as well as their mother S.W., were homozygous with respect to the genetic

marker Gm(4,5). The father (K.R.W.) of Y.W. was also homozygous Gm(4,5). The other relatives investigated were heterozygous with the genetic markers Gm(1) and Gm(4,5).

Specific humoral antibody formation. No antibodies to *Haemophilus influenzae* polysaccharide were found in sera from Y.W., R.W. and their mother S.W. (<1/4). Titres 1/16–1/32 were found in sera from all relatives examined and from ten normal children of

TABLE 2. Antibody response

	Y.W. 13 yr	R.W. 6 yr	S.W. mother	L.W. 10 yr
Isoagglutinins anti-B (titre) (all blood group A)	1/1	<1/1	1/2	1/32
Heteroagglutinins sheep cells (titre)	<1/2	<1/2	<1/2	1/8
Anti-*Haemophilus influenzae* polysaccharide (titre)	<1/4	<1/4	<1/4	1/32
Anti-teichoic acid (precipitation)	–	–	–	+

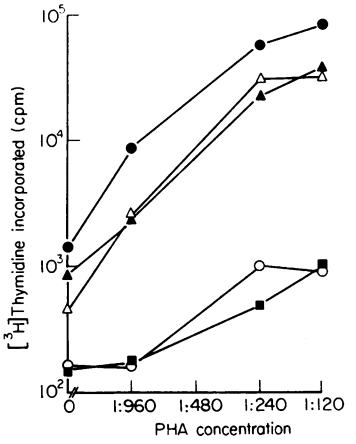

FIG. 2. Dose–response curve for culture of PHA stimulated peripheral lymphocytes of (○) Y.W , (●) R.W., (■) their mother S.W. and (△) (▲) controls. Y.W. and the mother S.W. do not respond normally.

the same age as the siblings and from a normal serum pool. No precipitating antibodies to teichoic acid were detectable in sera of the patients (Y.W. and R.W., and their mother S.W.) (Table 2). No heteroagglutinins against sheep blood cells (<1/4) could be shown in the patients, while the relatives and normal controls showed titres 1/8–1/16.

All three patients showed low titres of antibodies to measles antigen and the mother S.W. was also shown to have low titres of antibody against influenzae A. Rubella antibodies were

followed just after infection of patient Y.W., who showed significant antibody response of HAI antibodies to rubella from 1/10 to 1/40 and a rise of complement-fixing antibodies from <1/5 to 1/5. The mother showed HAI antibodies to rubella in a titre of 1/40. Antibodies to streptolysin-O were found (90–180 U/ml). No antibodies to human or rabbit γ-globulin were found by the Latex fixation test and by the Waaler–Rose test, respectively.

The Schick tests after diphtheria–tetanus toxoid vaccination of the patients were negative.

Cell-mediated immunity

Lymphopenia was found in the patient Y.W. with lymphocytes below 1500/mm^3. In R.W. peripheral lymphocytes were sometimes low and varied between 2200 and 4300/mm^3. There was often marked eosinophilia and monocytosis in the blood of both children.

In neither of the children did repeated X-ray examinations demonstrate the thymus. In the test of peripheral lymphocyte responsiveness to different doses of phytohaemagglutinin (PHA) the lymphocytes from Y.W. and the mother S.W. responded subnormally to all concentrations of PHA used while the lymphocytes of R.W. reacted normally (Fig. 2). Delayed hypersensitivity skin reaction to Trichophytin was negative and positive reactions were obtained with PPD, *Candida* and Varidase in Y.W. and the mother S.W. R.W. showed no reactivity to PPD, *Candida* and Trichophytin but was positive for Varidase. All three patients had been BCG vaccinated with no untoward reactions.

Treatment with gamma-globulin

Y.W. and R.W. were treated with a monthly injection of gamma-globulin in a dose of 0·1 g/kg body weight. The X-ray changes in the lungs of R.W. disappeared. R.W. has also required antibiotic therapy three times during the last 9 months because of otitis media and sinuitis. The patient Y.W. has also been treated continuously with ampicillin and there has been no progression of the lung changes.

DISCUSSION

The findings in the two children and their mother suggested an inherited IgG2 and IgG4 deficiency. This inheritance was a non-X-linked autosomal recessive. The deletions of the IgG2 and the IgG4 allelles were in all three patients associated with the Gm(4,5) allelle. This indicated an inherited abnormal gene complex. A structural gene defect could be suggested or there might be a regulatory gene defect with abnormally weak expression of normal structural genes. IgG subclass imbalances have been noted in immunodeficiencies and in patients with recurrent infections (Terry, 1968; Rivat *et al.*, 1969; Schur *et al.*, 1970; Yount *et al.*, 1970). The three patients described in the present paper showed quantitatively normal but qualitatively defective IgG with absence of the IgG2 subclass and IgG4 subclass. IgG2 normally constitutes about 20% and IgG4 about 4% of the normal IgG in serum. A total deficiency of IgG2 and IgG4 might leave the IgG value within the normal range.

No precipitating antibodies against teichoic acid were found in our patients' sera. This agrees well with the IgG2 deficiency, as antibodies to carbohydrate antigen such as teichoic acid, dextran and levan, have been reported to belong to the IgG2 subclass (Yount *et al.*, 1968). Furthermore, haemagglutinating antibodies to *Haemophilus influenzae* polysaccharide were lacking in sera from the patients, although they had had repeated *Haemophilus influenzae* infections. This might indicate that in normals these antibodies are of the IgG2 subclass.

The two siblings and their mother had strikingly low titres of isohaemagglutinins. Also the normally occurring heteroagglutinins against sheep cells were missing. These findings indicate a deficiency also of the IgM antibody response as isohaemagglutinins and Forssman antibodies are known to be IgM. It might therefore be assumed that both the IgM response as well as the IgG2 response to polysaccharide antigen were deficient. An inability to express IgM antibodies to some polysaccharide antigens is seen in the Wiscott–Aldrich syndrome. In this disease Cooper and coworkers (1968) suggested a defect in antigen recognition or antigen processing. Three patients with Wiscott–Aldrich syndrome were investigated with low levels of IgM and were found to have detectable IgG2.

Some humoral antibodies were shown, such as anti-streptolysin-O antibodies and anti-diphtheria toxoid antibodies revealed by negative Schick tests. Precipitating diphtheria toxoid antibodies have been found to belong to the IgG1 subclass (Yount *et al.*, 1968).

On the basis of the observed lymphopenia in the peripheral blood and poor uptake of [^{3}H]thymidine of peripheral lymphocytes after PHA stimulation Y.W. and the mother S.W. also exhibited a deficiency of cellular immunity. Their delayed skin reactions appeared to be the same. In R.W. cellular immunodeficiency was not so apparent with the normal lymphocyte response after PHA stimulation, but is suggested by negative skin tests to PPD, *Candida* and Trichophytin antigen. It is not possible to assess the influence of the long-standing chronic infections in Y.W. and the mother S.W. on the cellular immunological competence.

Malignant diseases are common in patients with certain types of immunodeficiency states such as ataxia teleangiectasia and Wiscott–Aldrich syndrome and appear even more frequently in the common variable form of immunodeficiency (Gatti & Good, 1970). Y.W. developed an epithelial tumour of the ovary with atypical cells and mitosis. The pattern of the cellular immune disorder found in our patients resembles that of Wiscott–Aldrich syndrome, where a progressive anergy of cellular immunity is noted.

The findings of the patients are consistent with an inherited disorder and constitute another example of different genetic mechanisms controlling the synthesis of each IgG subclass (Rivat *et al.*, 1970). A selective humoral immune deficiency with inability to synthesize antibodies to polysaccharide antigens, both IgM antibodies and IgG2 antibodies, was found. The patients also manifested impaired cellular immunity. The importance of early recognition of conditions of selective IgG subclass deficiency in patients with normal levels of IgG is stressed by the clear regression and clinical improvement following substitution therapy with gamma-globulin.

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